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# A Field Bioassay Approach to Assess the Toxicity of Insecticide Residues on Soil to Collembola

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**Abstract:** Field bioassays were conducted to assess the toxicity of three insecticides, chlorpyrifos, cypermethrin and pirimicarb, to four species of springtails, *Isotoma viridis*, *Isotomurus palustris*, *Folsomia candida* (Collembola: Isotomidae) and *Sminthurus viridis* (Collembola: Sminthuridae). Spray residues on two soil types (a sandy clay loam and a sandy soil) were obtained in the field, in the presence and absence of a wheat crop canopy, after spray application by a commercial tractor-mounted sprayer. Collembola were then confined for 24-h periods on the sprayed soils in a constant laboratory environment at 1, 2, 3, 8 and 15 days after treatment. Residual insecticide toxicity was compared between species, insecticides, soils and exposure conditions (crop or no crop) using the age of residue at which median mortality occurred ($DAT_{50}$). Cypermethrin and pirimicarb residues were of low toxicity, causing less than 10% mortality, whereas residues of chloryprifos were toxic to all four species of Collembola on both soil types and in both exposure treatments. Interspecific differences in collembolan susceptibility to chlorpyrifos residues gave the ranking (from most to least susceptible) *S. viridis* > *F. candida* > *Isotomurus palustris* > *Isotoma viridis*. Residues on the sandy soil were more toxic than those on the sandy clay loam. These results are discussed in terms of how field bioassay approaches may be used to determine pesticide residual toxicity to microarthropods. We conclude that field bioassays offer a feasible method for evaluating the toxicity of pesticides and the persistence of toxic effects on Collembola. Advantages and disadvantages of this method are considered.

Key words: *Isotoma viridis*, *Isotomurus palustris*, *Folsomia candida*, *Sminthurus viridis*, Collembola, chlorpyrifos, cypermethrin, pirimicarb, cereal crop, $DAT_{50}$

## 1 INTRODUCTION

A number of reviews have examined side-effects of pesticides on Collembola[1–4] but since the 1960s few European studies of pesticide effects have monitored these arthropods and even fewer have examined effects on individual collembolan species. Consequently, whilst responses of a broad range of beneficial invertebrate taxa to current widely used insecticides such as cypermethrin and pirimicarb are known, there is no published information on responses of Collembola to these chemicals in temperate arable cropping.[5] There is evidence that Collembola are more susceptible to pesticides than other routinely monitored arthropods in laboratory[6] and field studies.[5] Results from some recent long-term European farming systems experiments have indicated that pesticide regimes can cause prolonged adverse effects on collembolan populations in cereals,[7] arable rotations,[8] hops and vineyards,[9] but such long-term experiments are generally not capable of identifying effects of individual chemicals unless resulting population changes are substantial, e.g. following use of broad-spectrum organophosphorus insecticides.[10] For chemicals with more subtle effects, replicated within-season field trials may be the only feasible way of detecting effects of individual pesticides on non-target

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species. However, a review of recent studies monitoring pesticide effects on Collembola[5] noted some problems in comparing the results of different studies. These included differences in the species monitored as a result of faunal heterogeneity between sites, and a lack of standard methodologies in field experiments. Use of field bioassays would allow a greater degree of standardization in such experiments, making them more directly comparable, and would afford the experimenter control over the selection of study species. Field bioassays also provide a route whereby laboratory test systems (artificial soils, laboratory-cultured species) can be evaluated against field systems (natural soils, field-sampled insects) to determine the realism of artificial systems.

In this paper we have attempted to develop and test the feasibility of an *in-situ* field bioassay method for Collembola, and to use this approach to compare: (1) the toxicity of residues of three widely used insecticides to Collembola under realistic levels of residual exposure in a cereal crop; (2) susceptibilities of four collembolan species; and (3) the toxicity of spray residues on two different soil types.

## 2 MATERIALS AND METHODS

### 2.1 Experimental site

The experimental site was located in south-east England (51° 16′ N, O° 23′ E) at Mereworth in mid-Kent (TQ 660535). The 3·4-ha field in which the bioassays were carried out was one of four contiguous fields under winter wheat (*Triticum aestivum* L. cv. Hereward, drilled mid-October 1993). The bioassay field was selected as on of four fields already being used in a study to investigate effects of summer insecticide use on epigeal Collembola which provided data on the natural species composition and abundance of Collembola at the study site.

Each of the fields was divided into four adjacent areas to allow effects of four treatments to be compared (Fig. 1). In the bioassay field these areas were each *c.*0·8 ha. Examination of the density of crop stems (880–916 $m^{-2}$), the growth stage (g.s. 61–69, with 70% of plants at g.s. 65) and density of weeds in mid-June showed no differences between the treatment areas. The commonest weed species, *Urtica dioica* L. (common nettle), was patchily distributed in the field and estimates did not exceed 5% ground cover in 1·0 $m^2$ quadrats.

### 2.2 Experimental design

Within each of the four treatments (three insecticides and an unsprayed control) there were two levels of exposure (bioassays on soil with and without a crop canopy) and two soil types (field and Lufa 2·2). Comparisons of species, soils and exposure levels were replicated within each treatment at three locations, each *c.*30 m apart (Fig. 1). At each of these locations wheat plants were removed from an area of 4 $m^2$ to allow direct comparisons of exposure with and without a crop canopy. These represented a realistic range of exposure of Collembola to spray residues on soil, with bare soil a worst-case scenario, i.e. analogous to spray reaching the ground in tractor wheelings or areas of poor crop development.

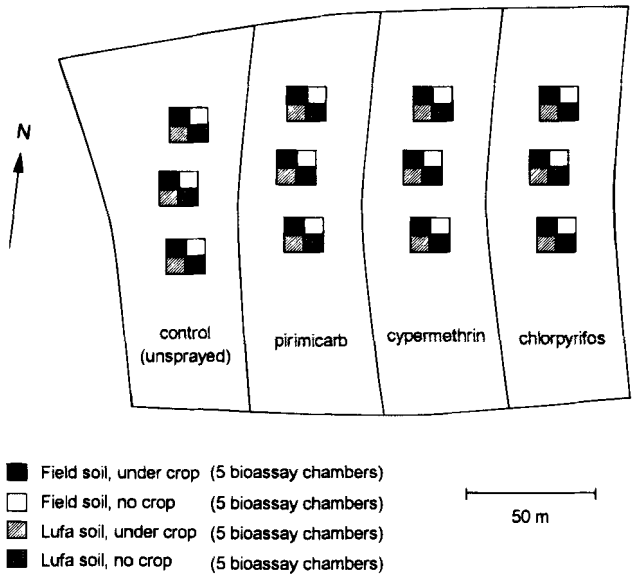


**Fig. 1.** Experimental plot layout and location of bioassay chambers.

Bioassay chambers were placed on the ground in the central *c.*0·5 $m^2$ of these cleared areas and in adjacent *c.*0·5 $m^2$ areas of undamaged crop. Bioassays on bare ground were separated from those under the wheat canopy by a *c.*0·5 m-wide buffer zone of intact crop plants. A total of 240 bioassay chambers was used, with five replicates for each pesticide, soil and exposure treatment comparison as shown in Fig. 1. All chambers, containing plastic inlays to prevent deposition on the chamber sides (see below), were placed on the ground in the field within 4 h prior to the insecticide spray applications. Approximately 30 min after the final spray application all chambers were removed from the field plots. The inlays were removed and lids were placed over the chambers before they were returned to the laboratory.

### 2.3 Choice of treatments

The four treatments comprised three insecticides and an unsprayed control. The insecticides were cypermethrin 100 g $litre^{-1}$ EC ('Ambush C'®; Zeneca), pirimicarb 500 g $kg^{-1}$ SG ('Aphox'®; Zeneca) and chlorpyrifos 480 g $litre^{-1}$ EC ('Spannit'®; PBI). These are widely

used insecticides: cypermethrin is currently the most extensively used insecticide active ingredient in UK arable crops, and in terms of the area of wheat treated in 1994, it accounted for 21% of the total insecticide use, pirimicarb 10% and chlorpyrifos 16%.[11] Cypermethrin and pirimicarb were chosen because no published work has evaluated the effects of these aphicides on Collembola.[5] In contrast, chlorpyrifos is known to have insecticidal activity against several species in the field[5] and in laboratory bioassays[12–14] and was used as a toxic standard for comparison with the other treatments. In this work cypermethrin and pirimicarb were applied as summer aphicide sprays against cereal and rose-grain aphids, *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Walker), and chlorpyrifos was used as a spray against orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin), with potential control also of aphids. The insecticides were applied according to their label recommendations; although chlorpyrifos is normally applied at ear emergence (growth stage 51 to 59), growers would use a later spray (as in this work; growth stage 61 to 69) if other nearby crops were susceptible and deemed to be at risk from midge attack.

### 2.4 Insecticide spray applications

The insecticides were applied in dry weather on 23 June 1994 between 0930 h and 1200 h BST using a Hardi LY 800 tractor-mounted 12-m boom sprayer with a 20-nozzle system of 110° flat fan (F110/1·59/3) nozzles. At an operating pressure of 2·2 bar a volume rate of *c.*200 litre $ha^{-1}$ was achieved with a tractor velocity of 6·9 km $h^{-1}$. Chemical application rates were 25, 140 and 480 g AI $ha^{-1}$ for cypermethrin, pirimicarb and chlorpyrifos respectively. To reduce the risk of cross-contamination, the insecticide applications were made in ascending order of their known toxicity to other non-target arthropods (pirimicarb, cypermethrin, chlorpyrifos) and the spray tank was rinsed thoroughly after each chemical was discharged. The wind direction was variable during the applications of pirimicarb and cypermethrin (3 to 5 km $h^{-1}$ with occasional gusts up to 9 km $h^{-1}$) and SE during the application of chlorpyrifos (3 to 5 km $h^{-1}$). The local screen temperature (measured *c.*0·3 km north-west of the centre of the field) increased from 20°C during the application of pirimicarb to 22°C during the application of chlorpyrifos; the maximum reached before bioassay chambers were removed from the field was *c.*24°C.

### 2.5 *In-situ* bioassay chambers

The *in-situ* soil bioassay method used was based upon that described in Wiles and Jepson.[15] This methodology was originally developed for testing pesticide toxicity to arthropods such as Carabidae, Staphylinidae, Coccinellidae and Linyphiidae and had not previously been used for Collembola. The bioassay chambers consisted of plastic containers (9 cm diameter × 6·5 cm high), the inside walls of which were coated with 'Fluon'® (PTFE) to prevent the test animals from climbing the chamber side. A 50 (±2) g sample of test soil (either field or Lufa) was placed in each bioassay chamber and compacted lightly using an 80-g weight. This ensured a firm and uniform surface for exposure. A plastic inlay consisting of an identical container with the bottom removed was placed in each chamber before spraying. After spraying, the inlays were removed, leaving the sides of the chamber above the soil free from spray deposits, and a lid was placed on each chamber during transfer to the laboratory.

### 2.6 Quantification of spray deposition at ground level

Spray deposition on bare soil and under the wheat canopy was quantified using strips (2·5 × 6 cm) of 'Teejet'® water-sensitive paper placed on the ground at the three replicate locations in each treatment area. Strips were also placed in the unsprayed area to determine if contamination from spray drift had occurred. After the spray application the paper strips were allowed to dry, carefully removed from the crop and wrapped individually in foil. Droplet deposition was measured on the strips in the laboratory using an IBAS image analysis computer (Kontron Ltd). Spray deposition rates ($\mu l\ cm^{-2}$) were estimated for each spray treatment using laboratory-prepared calibration curves.

### 2.7 Experimental soil types

The two soil types used in the bioassays were field soil from the experimental site and Lufa 2.2 soil. Lufa 2.2 soil is a commercially available, standard soil (Bezirksverband, Pfalz-Kasse Zahlstelle, 6720 Speyer, Germany) and was chosen because it differed markedly in its particle composition (Table 1) from the field soil, the field soil being classified as a sandy clay loam and Lufa 2.2 as a sandy soil.[16] The use of these two soil types permitted substrate-mediated differences in toxicity between a natural and a standard test soil to be investigated. Both soils had been defaunated (heated to 70°C for 2 h) and stored in a dark room at 5°C. Immediately prior to bioassay chamber preparation, samples of each were sieved through a 1-mm mesh sieve and made up to 50% of their respective water-holding capacities with distilled water (Table 1).

### 2.8 Selection and collection of test species

Large numbers of the lucerne-flea *Sminthurus viridis* L. (Collembola: Sminthuridae), *Isotomurus palustris*

TABLE 1
Test Soil Properties

| *Property*[a] | *Field soil* | *Lufa 2.2 soil* |
|---|---|---|
| Organic matter (%) | 7·1 | 4·5 |
| Clay (%) | 13 | 6 |
| Silt (%) | 20 | 4 |
| Sand (%) | 61 | 86 |
| pH (1 M KCl) | 6·7 | 5·9 |
| Moisture content (%) (after preparation) | 17·2 | 16·7 |

[a] Soil analysis: estimates of mean organic matter contents were made via a loss-on-ignition method in which 1-g samples of oven-dried soil were heated to 550°C for 2 h. Estimates of soil particle fractionation were made using a Bouyoucos hydrometer method.

(Müller) and *Isotoma viridis* Bourlet (Collembola: Isotomidae) were collected in June 1994 using a Ryobi suction sampler[17] from within cereal fields and grassy field margins adjacent to the experimental field site and from grassy verges within the grounds of the University of Southampton. Samples from both sites were combined and individuals of each species were tentatively identified and placed into separate containers. These species were chosen as they occur widely in agricultural land[5] and large numbers were available for the experimental work. The fourth test species, *Folsomia candida* Willem (Collembola: Isotomidae) was obtained from laboratory cultures held in the Department of Biology at the University of Southampton and was included for comparison as a standard laboratory test species.[18–20]

### 2.9 Maintenance of Collembola

Prior to the experiment all species were kept under controlled conditions of 20 (±2)°C and 16 : 8 h light : dark in clear plastic boxes containing a 1·5-cm-deep layer of moistened plaster of Paris impregnated with granulated charcoal (9 : 1 by weight). The three field-collected species were provided with granules of bakers' yeast, approximately 10 g of defaunated field soil and a small amount of grassy vegetation. *F. candida* was provided with bakers' yeast only. Individuals were randomly taken from each box for taxonomic verification under light microscopy (up to ×1000 magnification with phase-contrast where necessary). The individuals of *Isotomurus palustris* used in these experiments corresponded in coloration to the morphological forms *bimaculata* and *maculata sensu* Stach;[21] their characteristic pigmentation allowed them to be readily distinguished in most instars from the morphologically similar *Isotoma viridis*. Immediately prior to the experiments, individuals of *S. viridis*, *Isotomurus palustris* and *Isotoma viridis* were sorted according to the most abundant size class available for each species, in order to standardize the test animals as far as possible. *F. candida* were obtained from a synchronized culture to ensure uniformity of size. To quantify the size of the test organisms, the body length (excluding antennae and with the furcula retracted) was measured using an IBAS image analysis computer and fresh weights were recorded (Table 2).

### 2.10 Post-treatment assessments

In the laboratory, chambers were stored under controlled conditions of 20 (±2)°C and 16 : 8 h light : dark. Twenty-four-hour exposure bioassays were carried out at 1, 2, 3, 8 and 15 days after treatment. On each occasion 10 *S. viridis*, *Isotomurus palustris* and *Isotoma viridis* and 20 *F. candida* were released into each of three replicate chambers from the 16 treatment combinations (i.e. two exposure types, two soil types and four spray treatments). No food was provided during the 24-h exposure. Mortality was assessed after the 24-h period using a flotation technique. Approximately 100 ml of distilled water was added to each chamber and the soil was gently stirred. Surviving and dead Collembola could be observed and counted on the water surface. The stirring procedure was carried out twice and any Collembola still unaccounted for were assumed dead. Preliminary experiments to calibrate the flotation method had indicated that more than 95% of Collembola could be recovered using this method.

### 2.11 Statistical analysis

Mortality data for each species from the 24-h exposure bioassays were analysed by probit analysis for spray deposits of different ages to obtain the age of residue

TABLE 2
Size and Weight of Test Species

| *Test species* | *Mean body length (mm)* | *S.E. (mm)* | *Mean body weight (mg)* | *S.E. (mg)* |
|---|---|---|---|---|
| *S. viridis* | 1·02 | 0·11 | 0·07 | [a] |
| *F. candida* | 1·43 | 0·08 | 0·13 | [a] |
| *I. palustris* | 1·82 | 0·18 | 0·20 | [a] |
| *I. viridis* | 2·92 | 0·31 | 0·64 | 0·41 |

[a] Mean body weight obtained from total mass of 10 Collembola.

that gave a median lethal effect (i.e. the number of days after treatment during which 50% mortality occurred when insects were introduced to test chambers for a 24-h exposure period). Here we use the term $DAT_{50}$ (age of residue in days giving median lethal effect) to distinguish the estimates from the toxicological parameter $LT_{50}$ (median lethal effect time under continuous exposure). $DAT_{50}$ estimates were obtained from standard probit analysis and the intercepts and slopes of the probit lines were compared between species by maximum likelihood procedures[22] where data met the statistical requirements for the test. Pairwise comparisons were used to infer significant interspecific differences in Collembola susceptibility to insecticide residues. All response data were corrected for control mortality using Abbott's formula.[23]

## 3 RESULTS

### 3.1 Influence of crop cover on spray deposition and toxicity

Volumetric spray deposition rates did not differ significantly between the three chemical treatments ($F = 1{\cdot}4$; d.f. 2,66; $P > 0{\cdot}05$), indicating that spray application volumes were homogeneous in the experimental plots (Fig. 2). Soil under the crop canopy, however, received significantly less spray than bare soil ($F = 999{\cdot}7$; d.f. 1,66; $P < 0{\cdot}001$) (Fig. 2), with mean spray deposition rates varying from 1·91 to 2·12 $\mu l\ cm^{-2}$ and 0·11 to 0·13 $\mu l\ cm^{-2}$ on bare soil and soil from under the cereal crop canopy respectively. Exposure of Collembola to chlorpyrifos residues resulted in higher levels of mortality for individuals confined on the bare soil than those on soil from under the crop canopy for all species (Fig. 3), reflecting the different levels of chemical residues received by the soils. Laboratory determination of post-treatment soil moisture contents, however, indicated that differences in soil moisture levels may have influenced the toxicity of chemical residues to the Collembola, as soil from the fully exposed bioassay chambers (i.e. no crop) had a mean moisture content of 4·1%, whereas soil in chambers under the crop contained 15·8% moisture. Control mortality of Collembola

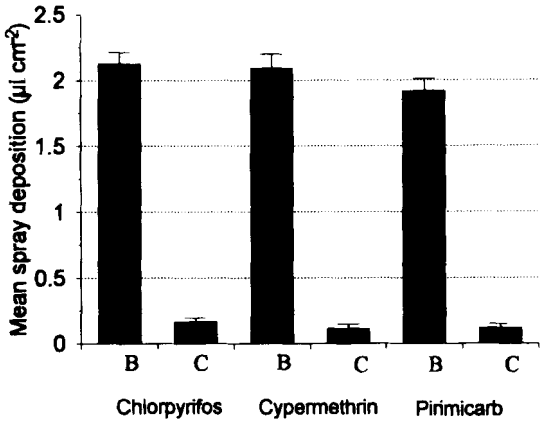


**Fig. 2.** Volumetric spray deposition in the experimental plots (bars indicate S.E.s). B ≡ bare soil without crop; C ≡ soil under crop.

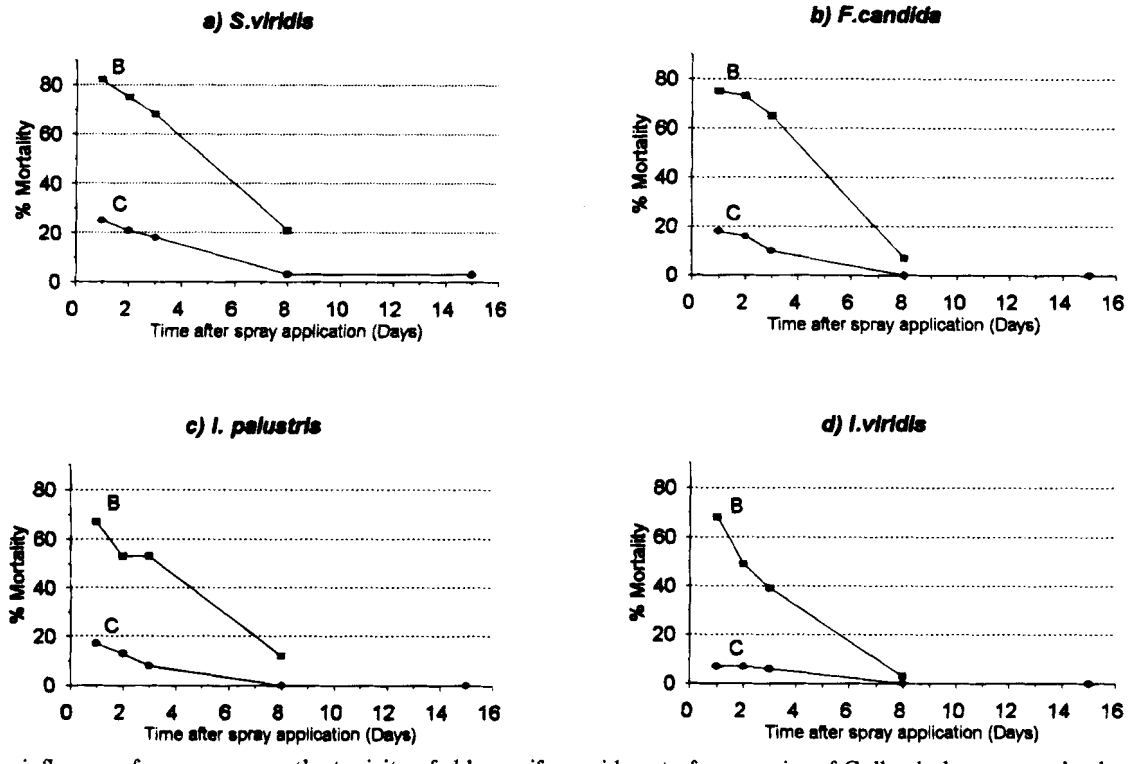


**Fig. 3.** The influence of crop cover on the toxicity of chlorpyrifos residues to four species of Collembola on a sandy clay loam soil. B ≡ bare soil without crop; C ≡ soil under crop. All data corrected for control mortality.

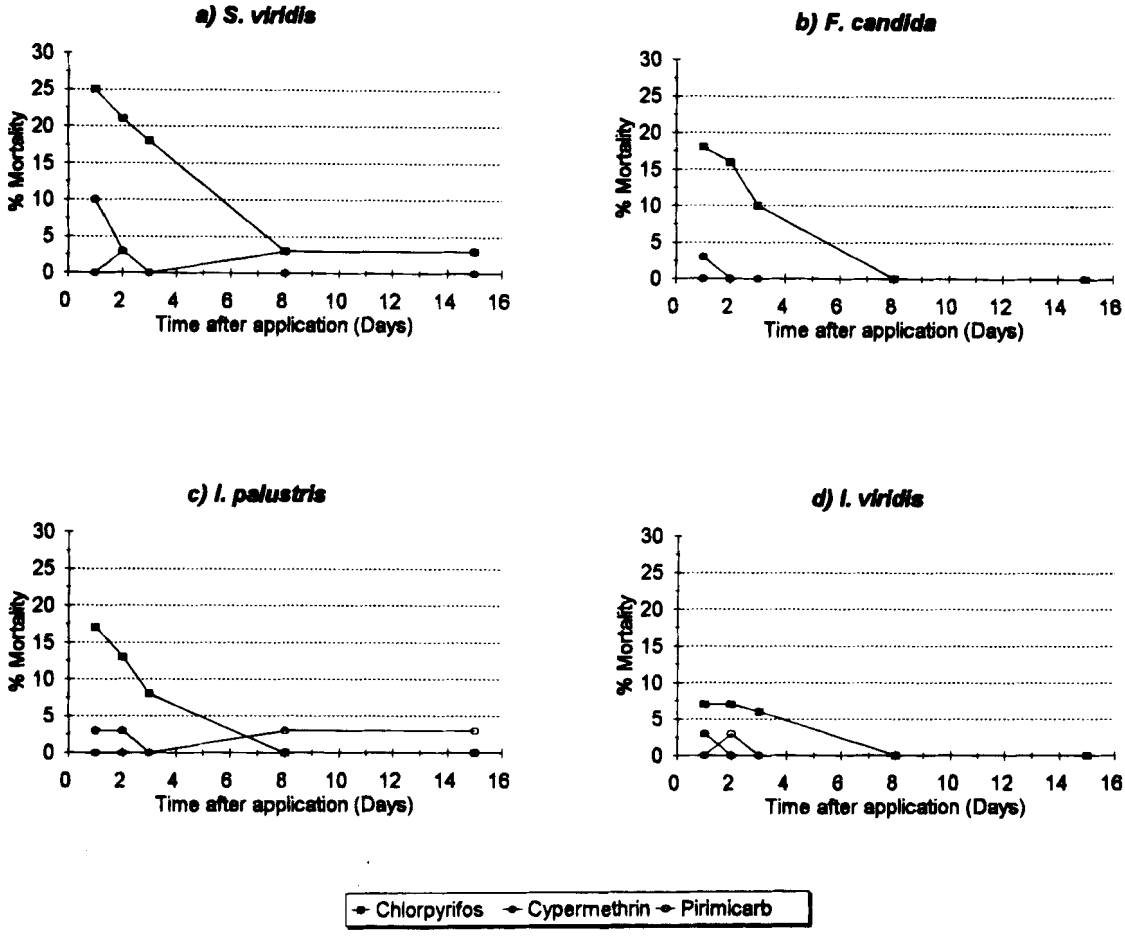


Fig. 4. The toxicity of chlorpyrifos, cypermethrin and pirimicarb residues to Collembola on a sandy clay loam soil under a cereal crop canopy. All data corrected for control mortality.

exposed on the former soil ranged from 12 to 23%, 28 to 45%, 30 to 37% and 23 to 37% for *S. viridis*, *F. candida*, *Isotomurus palustris* and *Isotoma viridis*, whereas control mortality of Collembola exposed on the latter varied from 0 to 6%, 0 to 5%, 0 to 3% and 0% respectively. The differences between the toxicities of chlorpyrifos residues to Collembola on soil with and without a crop canopy declined markedly over the few days after spray application and toxicity was negligible by eight days after treatment (Fig. 3).

### 3.2 Relative toxicity of chlorpyrifos, cypermethrin and pirimicarb to Collembola

Consistent patterns of initial toxicity to the four Collembola species were evident among the three chemicals (Fig. 4), giving a toxicity ranking (from most to least toxic) of chlorpyrifos > cypermethrin > pirimicarb. Initial levels of mortality on sandy clay loam field soil under the crop canopy 24 h after spray application varied from 7 to 25% for chlorpyrifos and 3 to 10% for

TABLE 3
Probit Statistics for the Toxicity of Chlorpyrifos to Collembola on Bare Field Soil

| *Test species* | *Slope (S.E.)* | *Detransformed $DAT_{50}$ (d) (95% F.L.)*[a] | *Initial mortality (%) (1 DAT)* |
|---|---|---|---|
| *S. viridis* | −2·8 (0·6) | 3·9 (3·0–5·5) a | 82 |
| *F. candida* | −3·3 (0·4) | 3·1 (2·7–3·8) ab | 75 |
| *I. palustris* | −2.3 (0·5) | 2·4 (1·6–3·4) bc | 67 |
| *I. viridis* | −3·2 (0·7) | 2·0 (1·4–2·5) c | 68 |

[a] Different letters indicate significant differences in susceptibility ($P < 0.05$). All data sets were homogeneous ($P > 0.05$) except *F. candida* ($P < 0.05$).

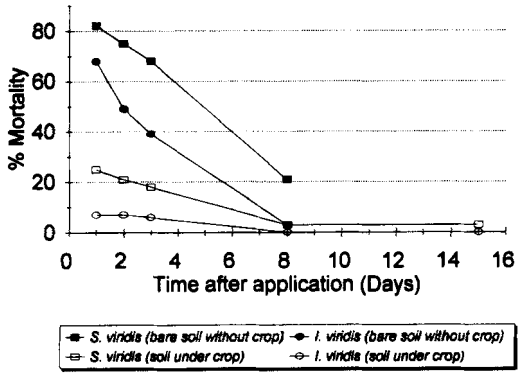


**Fig. 5.** The toxicity of chlorpyrifos residues to *Sminthurus viridis* and *Isotoma viridis* on a sandy clay loam soil in the presence and absence of a cereal crop canopy. All data corrected for control mortality.

cypermethrin, with no mortality observed for pirimicarb. Toxic effects of spray residues on Collembola were detected for two days after treatment with cypermethrin and for eight days with chlorpyrifos (Fig. 4).

### 3.3 Relative susceptibilities of species to chlorpyrifos

Interspecific comparisons of susceptibility to insecticide residues could only be made statistically for the most toxic compound, chlorpyrifos, on soils fully exposed to spray (Table 3). $DAT_{50}$ values varied from 3·93 days for *S. viridis* to 1·95 days for *Isotoma viridis*, with higher $DAT_{50}$ values indicating greater susceptibility because probit slopes were negative (Table 3). Pairwise comparisons of species susceptibilities indicated that *S. viridis* was significantly more susceptible than *Isotomurus palustris* and *Isotoma viridis* to chlorpyrifos residues. *F. candida* was of intermediate susceptibility but significant heterogeneity ($P < 0·05$) indicated a poor fit of probit line for this species. No significant interspecific differences were detected between the slopes of the fitted probit lines ($P > 0·05$) for any of the test species. The high initial toxicity of chlorpyrifos to *S. viridis* contrasted clearly with the lower initial toxicity to the least susceptible species, *Isotoma viridis*, under both exposure conditions (crop or no crop) (Fig. 5). $DAT_{50}$ values for Collembola exposed to chlorpyrifos on the sandy Lufa 2.2 soil varied between 6·47 days for *S. viridis* and 2·64 days for *Isotoma viridis* (Table 4), giving an identical ranking of species susceptibility to that on the field soil (*S. viridis* > *F. candida* > *Isotomurus palustris* > *Isotoma viridis*) and indicating that soil-mediated bioavailability did not affect interspecific susceptibility patterns.

**TABLE 4**
Probit Statistics for the Toxicity of Chlorpyrifos to Collembola on Bare Lufa 2.2 Soil

| *Test species* | *Slope (S.E.)* | *Detransformed $DAT_{50}$ (d) (95% F.L.)*[a] | *Initial mortality (%) (1 DAT)* |
|---|---|---|---|
| *S. viridis* | −3·2 (0·6) | 6·5 (5·0–9·8) a | 95 |
| *F. candida* | −3·4 (0·4) | 4·5 (4·1–5·9) ab | 88 |
| *I. palustris* | −2·3 (0·5) | 4·0 (2·9–6·1) bc | 79 |
| *I. viridis* | −3·3 (0·6) | 2·6 (2·0–3·4) c | 76 |

[a] Different letters indicate significant differences in susceptibility ($P < 0·05$). All data sets were homogeneous ($P > 0·05$).

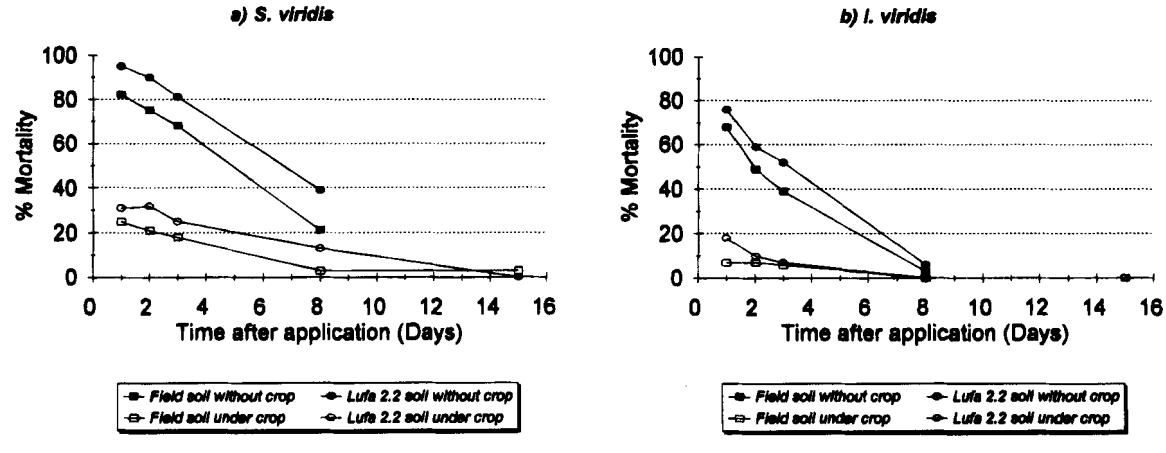


**Fig. 6.** The toxicity of chlorpyrifos residues to *Sminthurus viridis* and *Isotoma viridis* on two different soils. All data corrected for control mortality.

### 3.4 Comparison of the toxicity of chlorpyrifos to Collembola on field and Lufa soil

Chlorpyrifos residues in both exposure treatments (crop or no crop) were consistently more toxic to all four species of Collembola on the sandy Lufa 2.2 soil than on the sandy clay loam field soil. Residue toxicity to Collembola declined markedly on both soils during the bioassays; however the rate of decline was greater for residues on bare soil than for those obtained under a crop canopy (Fig. 6). Toxicity of chlorpyrifos residues was significantly higher ($P < 0{\cdot}05$) on the sandy Lufa 2.2 soil to the three most susceptible Collembola species (*S. viridis*, *F. candida* and *Isotomurus palustris*) than on the sandy clay loam field soil.

## 4 DISCUSSION

### 4.1 Relative toxicity of insecticides to Collembola

The ranking of insecticides in decreasing order of their initial toxicity to the Collembola test species (chlorpyrifos > cypermethrin > pirimicarb) is consistent with the general ranking organophosphate > pyrethroid > pirimicarb obtained from other studies with non-target arthropods. Pyrethroid insecticides are toxic to spiders, but for most other non-target taxa they are less harmful than organophosphorus chemicals in the laboratory[24,25] and field.[26–31] With some exceptions,[24,29,32] pirimicarb was usually the least toxic insecticide tested,[33] both in laboratory[24,25,34] and field studies.[26,31,35–38] Our work provides the first reported evidence of non-toxicity of pirimicarb to Collembola.

### 4.2 Relevance of the bioassays to the interpretation of risk of exposure in the field

The presence of a crop canopy greatly reduced spray penetration to ground level, with soil beneath the crop receiving 6–9% of the spray volume received by fully exposed soil. This level of deposition agrees closely with the results of other studies in winter wheat. For instance, Taylor & Andersen[39] and Cilgi & Jepson[40] found spray penetrations to soil of 9–13% at growth stages 45–85 and 9–14% at growth stages 56–73. The results provide a realistic range of exposure to residues on the ground in a wheat field, where local patches of soil in tractor wheelings and areas of poor crop growth would receive more spray deposition. Cilgi & Jepson[40] showed that crop senescence increased penetration of spray deposit as the density of lower foliage decreased, indicating that uneven ripening of a crop would also create heterogeneity in spray deposition on soil. Under such heterogeneous conditions of spray deposition in a wheat field it may be appropriate to estimate the best- and worst-case exposure scenarios by conducting bioassays on the least and most exposed areas of soil.

### 4.3 Comparison of soil types and influence of abiotic factors

Toxicity of chlorpyrifos residues to Collembola was consistently higher on the Lufa 2.2 soil than on the field soil (Fig. 6). The latter soil had a higher clay content (Table 1) and the results are consistent with observations in other studies that the toxic activity of organophosphorus insecticides to *F. candida*[41] and to larvae of the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber[42] was inversely related to the soil clay content. Clay-catalysed hydrolysis is a major route of dissipation of chlorpyrifos on dry soil surfaces[43] but there was no obvious difference between the two soil types in the rate of decline of chlorpyrifos toxicity (Fig. 6), presumably because neither soil was completely dry. Soils with a higher organic content have a greater sequestering capacity, which reduces the availability of insecticide for toxic effects[44] but this is unlikely to have been important in our work because differences in organic content between the two soils were small (Table 1).

The exposure treatment (crop or no crop) had a greater influence on the toxicity of chlorpyrifos residues than did soil type (Fig. 6), and the rate of decline of chlorpyrifos activity was lower on the soils exposed under the crop canopy. A potential problem in interpreting the results of the two bioassay exposure treatments is that bioassay soils taken from under the crop canopy had higher moisture contents than those from bare soil. The lower rate of decline of toxicity on the latter, moister, soil was unexpected because soil moisture usually increases the rate of insecticide degradation by encouraging microbial activity and chemical hydrolysis, and by increasing volatility;[45] this was seen by Monke & Mayo[42] when overall toxicities of insecticides to corn rootworm larvae were reduced in soils maintained at higher moisture contents. The lack of increase in chlorpyrifos degradation rate with increasing soil moisture observed in our study was also found by Getzin,[45] who suggested that destruction of the soil microbial population during autoclaving could be a plausible explanation. Another possible explanation for the lower decay rate of chlorpyrifos in soil exposed under the crop canopy in our study could be that adsorption sites were limited in availability. A greater proportion of the total deposition under the crop canopy would then have been adsorbed, leaving a smaller proportion available for hydrolysis and volatilization.

Differences in soil moisture content between the bare soil and that from beneath a crop canopy reflect an

important attribute of the microclimate under the crop foliage. In view of the importance of soil moisture in determining insecticide bioavailability,[45] it is desirable to preserve these differences during the laboratory incubation phase of the bioassays so that estimates of the best- and worst-case exposure scenarios are realistic.

The moisture content of soil is important not only from the point of view of pesticide chemistry but also because Collembola require a saturation deficit near zero for survival[46–48] and may migrate in response to unfavourable moisture conditions.[47] Susceptibility of species to low humidity has been shown to vary interspecifically, being greater for *Isotoma viridis* than for *Sminthurus viridis*.[46] In our work, the lower moisture content of the bare soil (no crop) bioassays led to increased collembolan mortality; this was greater for *I. viridis* (23–37%) than for *S. viridis* (12–23%), confirming the interspecific variability of these species to moisture stress. Differences in soil moisture between the exposure treatments (crop or no crop) reflect natural microclimatic effects so the moisture stress experienced by Collembola in the bioassays would be representative of realistic field circumstances. However, the bioassays enforced exposure of Collembola to humidity conditions they might otherwise have avoided in the field.

The persistence of chlorpyrifos toxicity has been shown to decrease with increasing incubation temperature in laboratory studies[13,43,45] and field applications made in hot weather were particularly susceptible to rapid dissipation.[49] Thompson & Gore[12] found the initial (*c.*24 h) residual contact toxicity of chlorpyrifos to *F. candida* to be higher at 24°C than at 13°C and attributed the increase with temperature to increased activity of the insects and decreased adsorption. In contrast, synthetic pyrethroid and organophosphorus insecticides had a higher mortality effect on carabid beetles at lower temperatures, a possible explanation being that the insects' detoxification ability decreased with temperature.[50] Our choice of laboratory incubation temperature (20 (±2)°C) approximated to the screen temperature at the time of spraying (20–24°C). The high initial toxicity but low persistence of chlorpyrifos is consistent with the results of Thompson & Gore[12,13] with *F. candida*. For bioassays to be realistic the incubation temperature should be varied to mimic changes in the ambient temperature at the field site after spraying. Although feasible, the benefits of such 'fine tuning' would have to be carefully considered in relation to the importance of other variables (wind direction and velocity, radiation, insolation, rainfall, etc.) which cannot be so easily manipulated in the laboratory.

### 4.4 Interspecific variation in insecticide toxicity

There was a considerable difference between *S. viridis*, the most susceptible species and *I. viridis*, the least susceptible species, in the mortality effect of the most toxic insecticide, chlorpyrifos (Figs 4, 5). Tomlin[51] observed considerable interspecific variation in susceptibility to fensulfothion between *F. candida* (highly susceptible) and *Onychiurus justi porteri* (not susceptible) and concluded that such variability precludes the extrapolation of toxicity data from one collembolan species to another. Interspecific variation in responses of epigeal Collembola species to pesticide use has also been observed in field studies[5] but the relative roles of differences in species' intrinsic susceptibilities and differences in their ecologies have yet to be resolved.

The most widely used Collembola in laboratory bioassays are edaphic species, especially *Folsomia* spp.[6,12,14,18–20,41,51–54] In comparison with epigeal Collembola, edaphic species posess morphological adaptations to a subterranean environment such as smaller appendages (antennae, legs and furcula), and they lack the dense covering of setae or scales found in some epigeal species. These morphological differences could influence the relative area of the insect cuticle available for direct contact with insecticide residues, but the overall size of insects and their behaviour will also be important. In our work an epigeal species collected from the field, *S. viridis*, was slightly more susceptible to chlorpyrifos than the laboratory test species *F. candida*, both in terms of initial mortality (1 DAT) and persistence of toxic effect ($DAT_{50}$), though these differences were not statistically significant (Table 3).

### 4.5 Intraspecific variation in insecticide toxicity

There was a clear relationship between the body size of the Collembola used and their susceptibility to the insecticide residues, the smallest species (*S. viridis*) being the most susceptible and the largest (*I. viridis*) the least susceptible (Tables 2, 3). It is plausible that interspecific differences in susceptibility were due to the different sizes of insects used, with smaller insects having a relatively larger area of cuticle that could be potentially exposed to residues or insecticide vapour. Unlike many other beneficial arthropods (e.g. Coleoptera and Arachnida), the collembolan cuticle is unsclerotized and acts as the major respiratory surface; *S. viridis* was unique among our test species in that it has a rudimentary tracheal system with spiracles and a well-developed abdominal appendage, the ventral tube,[46] whose exsertible vesicles have a role in water uptake and may considerably increase the body area. It is not known if these species-specific characteristics affected insecticide uptake. Even if body size was the major variable determining susceptibility, species comparisons in the bioassays would still have been valid and realistic because Collembola the same sizes as those used in the bioassays were present in the field at the time of spraying.

With the exception of the parthenogenic *F. candida* (all ♀♀), sex ratios were not determined in the bioassays. Sexual differences have been reported in the responses of Carabidae to pesticides[50] but rarely for Collembola, which are usually more difficult to sex when alive. In bioassay work the sex of dead specimens could be checked retrospectively after completion of bioassays, or subsamples of the source populations examined to determine the ratio of sexes present. A pronounced sexual dimorphism does occur in some Collembola of the family Sminthurididae, whose females may be considerably larger than males; bioassays on species of these Collembola would not only need to take into account the size differences between sexes, but also pre-reproductive behaviour in which the male is raised by the antennae of the female and is therefore out of contact with surface pesticide residues.

### 4.6 Collembola ecology and the toxic risk posed by insecticide residues

There is a general paucity of information on the ecology of Collembola in temperate arable ecosystems and consequently we lack the knowledge of how and where different species are exposed to pesticide residues. In additional field studies carried out at the bioassay field site, examination of 1600 wheat plants revealed only four individuals of *S. viridis*, although this species readily climbs crop plants to feed.[55] In contrast, *Isotomurus palustris*, which was not previously known to climb wheat plants, was present on the ears at a density of one per 10 plants (equivalent to *c.*96 insects $m^{-2}$). Quite apart from being at greater risk of direct exposure to sprays, climbing species such as these could be even more adversely affected by the deposited insecticide residues than the bioassays suggested because (1) they would be exposed to greater levels of residue higher in the canopy and (2) toxic activity of insecticide residues on wheat foliage may be more bioavailable and possibly persist for longer than those on the soil surface.[25] Edaphic species such as *F. candida* would only be exposed to spray deposition that reached the soil, but once in the soil, the decline in bioavailability of insecticide would be slower than on the soil surface.[49]

Residues and biological activity of chlorpyrifos may be detected for weeks after the application date,[13,42,43,45] but our results showed a rapid decline in the initial toxicity of its residues to Collembola. A rapid decline was also observed in the toxicities of deltamethrin and dimethoate to Coleoptera.[25] Given the high potential for temporal variability in the factors which determine bioavailability of residues (such as temperature and moisture), these results imply that the precise timing of an insecticide application may be crucial in determining the risk posed by toxic residues in the field.

### 4.7 Comparison of bioassay results with field data

Preliminary results of a Collembola monitoring study, using a Ryobi suction sampler, showed that 10 days after the insecticide applications (growth stages 73–75) mean catches of *Isotomurus palustris* ($\pm$ one S.E.; $n = 5$) in the bioassay field were: unsprayed 630·4 ($\pm$100·2); chlorpyrifos 10· 8($\pm$3·4); cypermethrin 389·2 ($\pm$32·5); pirimicarb 250·2 ($\pm$48·6). These figures are from one of four replicate fields and must be regarded as provisional, but they are consistent with the results of the bioassays in that considerably lower numbers of this species occurred in the chlorpyrifos-treated than in the unsprayed plot, whilst catches in the cypermethrin and pirimicarb plots were intermediate. Catches of *Isotoma viridis* showed a similar pattern (unsprayed 79·0 ($\pm$11·7); chlorpyrifos 0·2 ($\pm$0·2); cypermethrin 44·6 ($\pm$25·1)) but spatial heterogeneity precluded accurate estimation of this species in the pirimicarb treatment. Overall numbers of *S. viridis* were too low 10 days after spraying to permit a comparison between the insecticide treatments. Adverse effects of chlorpyrifos, but not of cypermethrin or pirimicarb, on field populations of *Isotoma viridis*, *Isotomurus palustris* and *S. viridis* have also been reported in other work.[5]

### 4.8 Field bioassay methodology—advantages and limitations

Bioassays investigating the relationship between mortality and time after exposure often use continuous exposure to a toxicant to determine the median lethal time ($LT_{50}$; the time after initial exposure at which 50% mortality of test animals occurs). Such continuous exposure of the test animals precludes the legitimate use of time as an independent variable[56] and thus cannot be used to determine the persistence or the rate of decay of a toxic effect. By exposing independent groups of test Collembola to residues of different ages (DAT) we were able to examine the time–toxicity relationship and compare this between different chemicals, species, soils and exposure conditions.

The general advantages and limitations of *in-situ* field bioassays were summarized by Wiles & Jepson.[15] Our field bioassay approach provided a workable method whereby intrinsic toxicity of pesticide residues to Collembola may be examined. Thompson & Gore[12,13] screened a wide range of insecticides against *F. candida* but few studies[51,52] have made interspecific comparisons, and studies have largely been confined wholly to the laboratory. Our method was suitable for at least three epigeal species collected from the field; this is important, because any attempt at improving our knowledge of the intrinsic susceptibility of Collembola to pesticides and its contribution to observed inter-

specific variation in the field will necessitate comparisons of different species. These field bioassays should allow test insects to be exposed to realistic levels of spray deposits on the soil, they can aid interpretation of the results of field trials, and allow a replicated experimental design at a relatively small spatial scale.

The field bioassay approach does have potential limitations, some of which could be overcome by modification of the method. The enforced exposure of insects to potentially unfavourable residues, moisture conditions or soil characteristics does not allow for effects of repellency or other behavioural changes.[53,54] Modification of the bioassay chambers to give test insects a choice between sprayed and unsprayed areas of soil would be relatively straightforward but the results from such two-dimensional systems may exaggerate the ability of Collembola to avoid or escape from toxic residues.[53] Use of defaunated soil in bioassays could underestimate the capacity of the soil to degrade pesticide through microbial activity, but Getzin[45] considered that for chlorpyrifos in a clay loam, autoclaving was acceptable as a pre-treatment because microbial degradation was relatively unimportant. In our work the broad similarity in the bioassay and field results supports that conclusion. Build-up of pesticide vapour and maintenance of adequate moisture are other potential limitations of bioassays using enclosed test chambers. The former problem could be ameliorated by passive or forced ventilation of the chambers whilst incorporation of a cotton wick[54] would improve soil moisture retention.

## 5 CONCLUSIONS

Field bioassays with Collembola are needed to investigate interspecific variation in susceptibility to pesticide residues because the lack of such information is currently a major impediment to the extrapolation of toxicity data from test species. The method described was suitable for three epigeal species which have not previously been used in toxicological assays. Results indicated high susceptibility of *Sminthurus viridis* to chlorpyrifos and a relative lack of toxicity of cypermethrin and pirimicarb to all species tested. Exposure of the plant-climbing species *S. viridis* and *Isotomurus palustris* could, however, have been underestimated by this method which focused on the soil residue route of exposure; knowledge of the ecology of test species is important for extrapolation of bioassay results to field populations. Given the potential complexity of exposure routes in the field, a number of bioassay approaches (soil residue, foliar residue, foliar oral) may be required for full assessment of the toxic risk of exposure of Collembola to pesticide residues in the field.

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